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Use of parvovirus capsid particles in the inhibition of hematopoietic progenitor cells.

The present invention relates to new uses of empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof. They may be used for the production of a medicament for the inhibition of hematopoietic progenitor cells, for administration to a patient for inhibition of hematopoietic progenitor cell growth, e.g. prior to stem cell transplatation, for inhibition of endothelial cell growth, or for treatment of hematological proliferative disorders of P antigen positive cells, e.g. polycytemia vera. These particles will preferably be included in a commercial package with written instructions for use.

Background of the invention

Human Parvovirus B19 is a significant pathogen associated with various clinical conditions, ranging from mild symptoms (erythema infectiosum) to more serious diseases in persons who are immunocompromised or suffer from hemolytic anemias. Hydrops fetalis and intrauterine fetal death are also well-known complications of parvovirus B19 infection during pregnancy (reviewed in 1).

Parvovirus B19 binds specifically to human erythroid progenitor cells (2) and inhibits hematopoietic colony formation by replicating in these cells (3). The suppression of hematopoietic cells has also been seen in bone marrow samples from infected individuals, resulting in transient anemia and, in rare cases, transient pancytopenia (4). Further, parvovirus B19 is known to cause bone marrow suppression in natural and experimental human infections (1). It has been suggested that the B19 non-structural protein, NS-1, induces apoptosis in erythroid lineage cells. Hitherto, it has been considered that the parvovirus B19 non-structural proteins are responsible for these effects in humans.

If it were possible, in a non-infecting way, to suppress hematopoiesis, this could be a new strategy when performing *in utero* transplantations. By suppressing the fetal stem cells of the recipient, prior to or at transplantation, the transplanted cells could get a competitive advantage over the native cells. The fetus seems in many respects to be an ideal host for the transplantation of hematopoetic stem cells, and successful transplantations have been performed in a number of different animal models (8-10). However, no successful cases have been reported in man, with the exception of transplants for immunodeficiency disease (11-14). These data and animal data on the W/Wv mouse model, which has a genetic stem cell deficiency (15), suggest that the donor cells need a competitive advantage over the recipient cells in order to achieve significant engraftment and long-term chimerism.

As no convenient methods are available for the culture of large quantities of native parvovirus B19 virus, which subsequently could be inactivated, genetically engineered expression systems for the production of parvovirus B19 antigens have been developed (5-7). Recombinant parvovirus B19 capsids, produced in a baculovirus system and composed of two structural polypeptides, the minor VP1 protein (83 kDa) and the major VP2 protein (58 kDa) (6). Parvovirus B19 VP1 and VP2 self-assemble and form virus-like particles. Parvovirus B19 capsids were recently evaluated in a clinical trial as a candidate vaccine against parvovirus B19 infection and were shown to induce a good neutralizing response without causing severe side effects (19). Electron microscopic analyses of the parvovirus B19 capsids showed them to be quite similar to plasma-derived virions (6). However, native, replicating virus and recombinant capsids probably show structural and conformational differences as well as differences in the presentation to the immune system. These capsids were previously shown to agglutinate human cells (17) and thus proven to have a receptor binding capacity to their natural target cell.

Description of the invention

The present invention provides the possibility of inhibiting hematopoietic stem cell colony forming assays with the parvovirus B19 capsids. We show that these capsids have an effect similar to that of native parvovirus, i.e. that hematopoietic stem cells are inhibited by these parvovirus B19 capsids, and, therefore, they may find use in a stem cell transplantation program.

One aspect of the invention is directed to the use of empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof, for the production of a medicament for the inhibition of hematopoietic progenitor cells. This inhibition may be effected in cell culture or in a patient.

The cellular receptor for parvovirus B19 is the P-antigen (2). The P-antigen is expressed on erythrocytes, erythroid progenitors, megakaryocytes, endothelium, kidney cortex, placenta, fetal myocardium (16) and pronormoblasts from fetal liver (18). Although parvovirus B19 can infect and lyse a wide range of human tissue and cell types expressing the P-antigen, only erythroid progenitor cells have been shown to be permissive for parvovirus B19 replication; it is also known that the virus requires mitotically active cells for its own replication. The effect of parvovirus B19 infection on hematopoietic cells in vivo has been studied in hematologically normal persons. They show transient anemia and usually absence of reticulocytes, as well as varying degrees of neutropenia and thrombocytopenia. In experimental infection of normal human volunteers, bone marrow samples revealed a severe

loss of erythroid precursor cells and a loss of both erythroid and myeloid progenitors in the peripheral blood.

In an embodiment of this aspect of the invention the medicament is a medicament for the inhibition of endothelial cell growth.

In another embodiment the medicament is a medicament for the treatment of hematological proliferative disorders of P antigen positive cells, e.g. polycytemia vera.

In an additional embodiment the medicament is a medicament for pretreatment of a patient prior to stem cell transplantation. In a preferred embodiment the patient is a fetus.

Non-myeloablative conditioning prior to postnatal stem cell transplantation is a recent development (20,21). Such protocols are less toxic to the patients than high-dose chemo-radiotherapy. However, complete donor hematopoietic chimerism may not be achieved to the same extent with non-myeloablative therapy. Using such protocols, it is now possible, according to the invention, to administer parvovirusB19 capsid to the patient prior to stem cell transplantation and thereby inhibit recipient hematopoiesis and promote donor cell engraftment.

Another aspect of the invention is directed to a method of treating a patient for inhibition of hematopoietic progenitor cell growth in said patient, comprising administration to said patient of a P-antigen blocking amount of empty, noninfectious, recombinant parvovirus capsid particles , or P-antigen blocking parts thereof.

Yet another aspect of the invention is directed to a method of treating a patient for inhibition of endothelial cell growth in said patient, comprising administration to said patient of a P-antigen blocking amount of empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof.

Still another aspect of the invention is directed to a method of treating a patient suffering from hematological proliferative disorders of P antigen positive cells, e.g. polycytemia vera, comprising administration to said patient of a P-antigen blocking amount of empty, noninfectious, recombinant parvovirus capsid particles , or P-antigen blocking parts thereof.

A further aspect of the invention is directed to a method of treating a patient for inhibition of hematopoietic progenitor cell growth in said patient prior to stem cell transplantation, comprising administration to said patient of a P-antigen blocking amount of empty, noninfectious, recombinant parvovirus capsid particles , or P-antigen blocking parts thereof. In a preferred embodiment the patient is a fetus.

The administration rout is e.g. intravascular, intramuscular, intradermal or intravenous. Excipients and/or diluents commonly used for the formulation of such preparations may be used. Reference is made to the US and European Pharmacopoeia for guidance.

The required P-antigen blocking amount of empty, noninfectious, recombinant parvovirus capsid particles or P-antigen blocking parts thereof will be decided based on the age, weight and clinical condition of the patient. Recommendations will be given in the commercial package including the parvovirus capsid particles or the P-antigen blocking parts thereof . The recommendations are given based on experimental data.

Thus, another aspect of the invention is directed to a commercial package containing empty, noninfectious, recombinant parvovirus capsid particles , or P-antigen blocking parts thereof, and written instructions for dosage and administration to a patient for hematopoietic progenitor cell growth inhibition, instructions for dosage and administration for hematopoietic progenitor cell growth inhibition in a patent prior to stem cell transplantation to said patient, such as a fetus, instructions for dosage and administration to a patient for endothelial cell growth inhibition and/or instructions for dosage and administration to a patient suffering from hematological proliferative disorders of P antigen positive cells, e.g. polycytemia vera.

Experiments

Colony formation assays were performed using fresh human fetal liver cells, umbilical cord blood cells and adult bone marrow cells. When recombinant parvovirus B19 empty capsids were added to these cells, a reduction was seen in the colony formation of BFU-E (burst forming unit-erythroid), CFU-GM (colony forming unit-granulocyte, macrophage) and CFU-GEMM (colony forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte) cells in an 11-day assay system. The colony formation of the precursor cells was rescued by preincubating parvovirus B19 capsids with anti-B19 monoclonal antibodies or with parvovirus B19 IgG positive human sera, prior to adding the mixture to the cells.

Further evidence that the inhibition of colony formation was indeed mediated by the parvovirus B19 capsids was obtained by using monoclonal antibodies against the cellular receptor for parvovirus B19, the P-antigen. When fresh fetal liver cells were preincubated with monoclonal antibodies against the P-antigen, prior to adding parvovirus B19 capsids, the inhibition of colony formation was reduced. Moreover, when a monoclonal antibody was used against the P_1 antigen, which is not involved in binding of parvovirus B19 to the cell surface, there was no protection. These effects are similar to those reported when

native virus was added to erythroid progenitor cells (2) and imply that the same P-antigen structure is used in both cases.

Methods

Donor cell preparation

Human fetuses 6-12 weeks of gestational age were obtained from legal abortions; the patients had volunteered to donate fetal tissue. The study was approved by the ethics committee at Huddinge Hospital. Gestational age was estimated according to specific anatomical markers and is given as menstruational age. Abortions were performed with vaccum aspirations. Fetal liver was dissected under sterile conditions, placed in a sterile tube containing RPMI1640 and disintegrated by passage through a vinyl mesh to form a single cell suspension. Nucleated cells were then washed three times, counted and diluted in culture medium.

Umbilical cord blood samples were obtained immediately after vaginal delivery from normal births. Samples of adult bone marrow were obtained from healthy allogeneic donors. Suspensions of fresh cells were heparinized and diluted in 0.9% NaCl and separated on Lymphoprep (Nycomed, Parma, Oslo, Norway) for gradient centrifugation at 2000 rpm for 20 min. Cells were carefully removed with a Pasteur pipette, washed three times in 0.9% NaCl, counted and diluted in culture medium.

Immunologic reagents

Recombinant parvovirus B19 empty capsid particles (6) were a gift from MedImmune (Gaithersburg, MD, USA). These capsids were produced in a recombinant baculovirus-insect cell (*Spodofera frugiperda*) expression system, previously described (6). Recombinant human papilloma virus capsids (HPV6) and Cottontail rabbit papillomavirus (CRPV) capsids were a gift from Dr. J. Dillner, Karolinska Institute, Stockholm, Sweden. These capsids had been produced in similar baculovirus-insect cell systems in the laboratories of Dr. J. Schiller, National Cancer Institute, USA and Dr. R. Kirnbauer, University of Vienna, Austria, respectively. Anti-parvovirus B19 monoclonal antibody (MAB8292) was purchased (Chemicon AB, Malmö, Sweden), as was anti-P_1 (Seraclone) monoclonal antibody (Labdesign, Stockholm, Sweden). Anti-P monoclonal antibody (CLB-ery-2) (16) was a gift from Dr. de Jong and Dr. von dem Borne (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). All monoclonal antibodies were mouse IgM except anti-parvovirus B19 which was IgG. Parvovirus B19 IgG positive (parvovirus B19 IgM negative) sera were obtained from two asymptomatic individuals in our laboratory.

Colony formation

A commercial kit "Stem cell CFU kit" (GIBCO BRL, Life Technology Inc., NY, USA) was used to study colony formation. This semi-solid system mimics the extracellular matrix produced by stromal cells. The components included are: Iscove's modified Dulbecco's medium, modified fetal bovine serum, methylcellulose, 2-mercaptoethanol, conditioned medium and erythropoietin. Colonies were identified as BFU-E (burst forming unit-erythroid cells) with densely packed hemoglobonized cells, CFU-GM (colony forming units-granulocytes, macrophages) with arrangements of non-hemoglobinized cells, and CFU-GEMM (colony forming units-granulocytes, erythroid cells, macrophages, megakaryocytes) with hemoglobinized cells and small and large peripheral cells.

Virus capsids were diluted in buffer (20 mM Tris, 0.5M NaCl, pH 8.5) and 30 µL of each dilution was added to $25x10^3$ fetal cells ($50x10^3$ for postnatal cells) in 100 µL of culture medium and incubated for 1 hr in +4 °C. The mixtures were then transferred to incubation dishes and culture medium was added to a final volume of 0.5 mL per well. The cells were incubated for 11 days in a humidified atmosphere at 5% CO_2, and were then scored for BFU-E, CFU-E and CFU-GEMM derived colony formation.

In additional experiments, either parvovirus B19 capsids or fetal cells were preincubated with antibodies prior to the incubation of the cells for 11 days:

a) 25 µL of either parvovirus B19 IgG positive serum or anti-parvovirus B19 monoclonal antibody (MAB8292) were preincubated with 25 µL of parvovirus B19 capsids for 2 hrs at +4°C. The mixtures were then added to cells as described above.

b) 25 µL of either anti-P (CLB-ery-2) or anti-P_1 (Seraclone) monoclonal antibody was added to $25x10^3$ cells suspended in 100 µL of medium. Cells and monoclonal antibody were preincubated for 1 hr at 4°C. The mixtures were washed twice in cold culture medium prior to adding parvovirus B19 capsids to the cells as described above.

All assay sets included triplicates of each serum, monoclonal antibody or capsid dilution. Triplicates of medium control (no added capsid or antibody) and triplicates of capsid control (no added antibody) were included in each experiment. As fresh human cells were used, on each occasion from different donors, the total number of colony counts varied between experiments. Appropriate controls ("capsid only") are therefore shown in the figures. Means of colony counts from the triplicates in representative experiments are given. Colony counts in the medium control were considered to be 100% in the respective experiments.

Results

Effect on fetal liver cell colony formation by virus capsids

Colony formation of BFU-E, CFU-GM and CFU-GEMM was assayed by culturing fresh fetal liver cells in culture medium in an 11-day standard assay. The resulting colony counts are expressed as percentage of the medium control. The capsids were shown to inhibit colony formation of all cells (BFU-E, CFU-GM, CFU-GEMM) (Table 1).

Recombinant papillomavirus capsids (Cottontail rabbit papillomavirus and human papillomavirus type 6) were included as control in the culture of fetal liver cells. These capsids are structurally similar to parvovirus B19 capsids but do not use the same receptor as parvovirus B19. The papilloma virus capsids had no effect on colony formation of any cell type (as tested in the range 0.01 - 100 μg/ml) (data not shown).

Neutralization of the B19 capsids

Parvovirus B19 capsids were preincubated with anti-B19 monoclonal antibody (MAB8292) prior to adding the mixture to fetal liver cells for the 11-day incubation period. Although a relatively high concentration of parvovirus B19 capsids (7 μg/ml, compare Table 1) was used, the anti-B19 monoclonal antibody (20μg/mL) was able to completely block the inhibitory effect on BFU-E and reduce the effect on CFU-GM and CFU-GEMM colony formation (Table 2A).

Two parvovirus B19 IgG positive sera from asymptomatic individuals were also tested for their neutralizing effect on the capsids prior to incubating the mixture with fetal liver cells. Parvovirus B19 capsids (0.14 μg/mL) alone inhibited BFU-E to 18% of the medium control. When parvovirus B19 capsids were preincubated with serum 1 (1:10 dilution), the inhibition was reduced (Table 2B). The neutralizing capacity of serum 2 was lower.

Blocking of the P-antigen on fetal liver cells

Fetal liver cells were preincubated with anti-P monoclonal antibody (CLB-ery-2). By blocking the parvovirus B19 cellular receptor with this monoclonal antibody, the inhibitory effect of the parvovirus B19 capsid was reduced by at least 25% for the different cells (BFU-E, CFU-GM, CFU-GEMM) (Table 3). Using a control monoclonal antibody to the P_1 antigen (Seraclone; not involved in parvovirus B19 binding), no effect on colony formation was seen compared with the parvovirus B19 capsid control.

Colony formation by umbilical cord blood and adult bone marrow stem cells

The inhibitory effect of parvovirus B19 capsids on colony formation was also tested using fresh stem cells derived from cord blood and adult bone marrow samples. The inhibitory effect was comparable to cultures of fetal liver cells (Table 4). Neutralization of the

parvovirus B19 capsids with anti-parvovirus B19 monoclonal antibody (MAB8292) in the same concentrations as in Table 2A was also comparable to cultures of fetal liver cells (data not shown).

The obtained results concerning the inhibitory effect of parvovirus B19 capsids on colony formation suggest that they can be used to inhibit hematopoesis in recipients prior to *in utero* stem cell transplantation. An approach in fetal stem cell transplantations would be to treat the recipient with parvovirus B19 capsids to suppress stem cell activity and thereby increase the donor-to-recipient cell ratio. In a previous study on tissue distribution of stem cells in the human fetus we estimated that a fetal transplantation with 5×10^7 cells in the second trimester will lead to a donor-to-recipient ratio of approximately 1:1000 - 1:10000. It is highly questionable whether such a low ratio in hematopoietic organs would be sufficient to give the transplanted cells a competitive edge over the native stem cells (14). Furthermore, it would be possible to pretreat donor cells with anti-P monoclonal antibodies to protect them from suppression by the capsids, thereby giving these cells an even more favourable status.

Table 1. Colony-forming unit assay of fetal liver cells. The cells were preincubated with dilutions of the parvovirus B19 capsids prior to the 11 d culture.

	Colony counts (% of medium control)		
Parvovirus B19 capsid (µg/mL)	BFU-E	CFU-GM	CFU-GEMM
70	22%	14%	31%
0.7	39%	54%	63%
0.007	79%	95%	94%
Medium (=100%), counts	95	37	16

Table 2. Colony-forming unit assay of fetal liver cells. The cells were preincubated with the following reagents prior to the 11 d culture.

A

Parvovirus B19 capsid (7 µg/mL) + dilutions of Anti-parvovirus B19 mab (µg/mL)	Colony counts (% of medium control) BFU-E	CFU-GM	CFU-GEMM
20	>100%	74%	67%
2	69%	35%	45%
0.2	52%	21%	19%
0.02	52%	30%	21%
capsid only	43%	30%	21%
Medium (=100%), counts	114	66	42

B

Parvovirus B19 capsid (0.14 µg/mL) + dilutions of two human parvovirus B19 IgG positive sera	Colony counts (% of medium control) BFU-E	CFU-GM	CFU-GEMM
Serum 1, 1:10	70%	78%	90%
Serum 1, 1:100	25%	23%	40%
Serum 2, 1:10	48%	57%	57%
Serum 2, 1:100	17%	27%	67%
capsid only	18%	17%	63%
Medium (=100%), counts	157	81	30

Table 3. Colony-forming unit assay of fetal liver cells. The cells were preincubated with the following reagents prior to the 11 d culture

Parvovirus B19 capsid (0.14 µg/mL)	Colony count (% of medium control)		
+ of Anti-P mab (titer)	BFU-E	CFU-GM	CFU-GEMM
1:5	51%	39%	93%
1:500	23%	10%	43%
capsid only	18%	17%	63%
Medium (=100%), counts	157	81	30
Parvovirus B19 capsid (0.14µg/mL)			
+ of Anti-P_1 mab (µg/mL)			
400	25%	20%	50%
4	17%	22%	47%
capsid only	18%	17%	63%
Medium (=100%), counts	157	81	30

Table 4. Colony-forming unit assay of cord blood and adult bone marrow cells, respectively. The cells were incubated with dilutions of B19 capsid (µg/mL) prior to the 11 d culture.

	Colony counts (% of medium control)		
Parvovirus B19 capsid (µg/mL)	BFU-E	CFU-GM	CFU-GEMM
Cord blood cells			
7	10%	54%	43%
0.7	33%	62%	43%
0.07	49%	72%	50%
0.007	57%	67%	70%
0.0007	84%	79%	93%
Medium (=100%), counts	134	39	30
Bone marrow cells			
7	18%	36%	6%
0.7	43%	45%	28%
0.07	63%	41%	44%
0.007	76%	80%	78%
0.0007	86%	77%	78%
Medium (=100%), counts	134	39	30

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Claims

1. Use of empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof, for the production of a medicament for the inhibition of hematopoietic progenitor cells.

2. Use according to claim 1, wherein the medicament is a medicament for the inhibition of endothelial cell growth.

3. Use according to claim 1, wherein the medicament is a medicament for the treatment of hematological proliferative disorders of P antigen positive cells, such as polycytemia vera.

4. Use according to claim 1, wherein the medicament is a medicament for pretreatment of a patient prior to stem cell transplantation.

5. Use according to claim 4, wherein the patient is a fetus.

6. Method of treating a patient for inhibition of hematopoietic progenitor cell growth in said patient, comprising administration to said patient of a P-antigen blocking amount of empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof.

7. Method of treating a patient for inhibition of endothelial cell growth in said patient, comprising administration to said patient of a P-antigen blocking amount of empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof.

8. Method of treating a patient suffering from hematological proliferative disorders of P antigen positive cells, such as polycytemia vera, comprising administration to said patient of a P-antigen blocking amount of empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof.

9. Method of treating a patient for inhibition of hematopoietic progenitor cell growth in said patient prior to stem cell transplantation, comprising administration to said patient of a P-antigen blocking amount of empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof.

10. Method of treating according to claim 9, wherein said patient is a fetus.

11. Commercial package containing empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof, and written instructions for dosage and administration to a patient for hematopoietic progenitor cell growth inhibition, instructions for dosage and administration for hematopoietic progenitor cell growth inhibition in a patent prior to stem cell transplantation to said patient, such as a fetus, instructions for dosage and administration to a patient for endothelial cell growth inhibition and/or instructions for dosage and administration to a patient suffering from hematological proliferative disorders of P antigen positive cells, such as polycytemia vera.

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Abstract

Use of empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof, for the production of a medicament for the inhibition of hematopoietic progenitor cells, inhibition of endothelial cell growth, treatment of hematological proliferative disorders of P antigen positive cells, such as polycytemia vera, and pretreatment of a patient, e.g. a fetus, prior to stem cell transplantation, is described.

Further, a method of treating a patient for inhibition of hematopoietic progenitor cell growth, for inhibition of endothelial cell growth, hematological proliferative disorders of P antigen positive cells, e.g. polycytemia vera, or inhibition of hematopoietic progenitor cell growth in said patient, e.g. a fetus, prior to stem cell transplantation, is described.

Additionally, a commercial package containing empty, noninfectious, recombinant parvovirus capsid particles, or P-antigen blocking parts thereof, and written instructions for use, is disclosed.